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STUDY OF THE EFFECTS OF URETHANE, SELENOCYSTINE AND SODIUM MONOHYDROGEN ARSENATE ON CROSSING-OVER IN *DROSOPHILA MELANOGASTER*

by

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled "Study of the Effects of Urethane, Selenocystine and Sodium monohydrogen arsenate on Crossing-over in *Drosophila melanogaster*" submitted by Zia Uddin Ahmed in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

A study of the effects of urethane, selenocystine and sodium monohydrogen arsenate on crossing-over in the X-chromosome of *Drosophila melanogaster* reveals that:

(1) Urethane causes a general increase in the frequency of crossing-over. The urethane curves show a concentration optimum for urethane at 3—10 mM.

(2) Selenocystine significantly alters the frequency of crossing-over. The selenocystine curves are doubly inflected with a 'peak' at 2 μM Se and a 'trough' at 10 μM Se.

(3) Sodium monohydrogen arsenate seems to cause a moderate increase in crossing-over particularly in segment 1 while the effect is much less marked in segments 2 and 3.

(4) High level of urethane (10 mM) interacts synergistically with all levels of selenocystine in altering the frequency of crossing-over while the interaction between low level of urethane (1 mM) with high level of selenocystine (10—50 μM) is antagonistic in nature.

(5) The interaction of urethane and sodium monohydrogen arsenate is predominantly antagonistic.

(6) Double exchange curves involving segments 1 and 3 show strong inflectional agreement with the single exchange curves for segment 1 implying an influence of segment 1 on the nature of the $DE_{1,3}$ curves.

(7) A lethal effect of 25 mM urethane on the male flies has been noted.

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INTRODUCTION

The process of crossing-over is sensitive to a variety of extrinsic factors including manipulation of cellular environment by chemical treatments. A number of studies have been reported on the effects of such chemical treatments as ions and inhibitors of DNA and protein synthesis on crossing-over. In general, these studies have not contributed significantly to an understanding of the mechanism of crossing-over.

Nevertheless, several studies and suggestions have been made relating the effects of ions on crossing-over to the hypothetical chromosome structure proposed by Mazia (1954) who viewed the chromosome as an aggregate of DNA and protein held together by divalent cations. Such studies, however, have not led to any definite conclusion regarding how the ions affect the process of crossing-over.

Ting and Walker (1969) found significant effects of selenocystine on crossing-over in the X-chromosome of *Drosophila melanogaster* and suggested that these effects may have resulted from selenium incorporation in the chromosomal protein with the resultant alterations of its structural characteristics causally related with DNA-breakage. Walker and Bradley (1969) extended this study to include the effects of sodium monohydrogen arsenate and the interacting effects of these two chemicals on crossing-over and offered a hypothesis relating the arsenate effect to its possible incorporation into the DNA.

The present study is a follow-up of the studies performed by Ting and Walker (1969) and Walker and Bradley (1969). This time, a third chemical, namely urethane, has been included in the study. The independent effects of urethane, selenocystine, and sodium monohydrogen arsenate and urethane-selenocystine and urethane-sodium monohydrogen arsenate interactions on

crossing-over have been investigated. It is recognized that the effect of manipulation of the cellular environment on crossing-over by such chemicals as those used in the study may be indirect.

REVIEW OF LITERATURE

In 1911 Morgan demonstrated exchange of markers and soon afterwards Morgan and Cattel (1912) proposed the term 'crossing-over' to describe the phenomenon which gives rise to new gene blocks. Despite the tremendous volume of work in this area, the mechanism underlying this process remains far from clear. However, with the identification of DNA as the genetic material and the spectacular progress in recent years in the area of molecular biology, attempts have been made to explain the phenomenon in molecular terms. Although this has considerably advanced our understanding of the process, for a more complete understanding of the mechanism it would be desirable to have a clearer picture of the chromosome of higher organisms as a nucleoprotein aggregate. Another area of controversy is the discovery of certain features in intragenic crossing-over which are not detectable in intergenic crossing-over.

Chiasmata and Crossing-over

Janssens (1909) discovered the chiasmata and it was immediately recognized that such structures could have broad implications in the phenomenon of crossing-over. He postulated the Chiasmatype Theory to account for these structures. The theory states that exchange of chromosome strands between two homologues takes place at the four-chromatid stage and the exchange gives rise to the cross-shaped structures, causing simultaneously the recombination of linked genes. The hypothesis which links the chiasma with genetic recombination possesses features which can satisfactorily explain the cytological and genetic phenomenon of meiosis in relation to crossing-over. The chiasmatype theory implicates a breakage-reunion mechanism in the origin of the chiasmata. However, it does not specify

the cause of strand breakage.

There was some controversy whether the chiasma might be the cause (Sax, 1932) or the consequence (Belling, 1928) of crossing-over. Subsequent findings favored Belling's interpretation, which suggests the existence of a 2:1 ratio between chiasma number and recombination frequency. This necessarily has to be so because each exchange event involves only two of the four chromatids of a tetrad. It was thus established that chiasmata are the consequence of previous crossing-over. Soon it was further demonstrated that crossing-over is associated with chromosomal exchange (Creighton and McClintock, 1931; Stern, 1931) and from the results of tetrad analysis of *Neurospora crassa*, that only two of the four strands take part in a single cross-over (Lindegren, 1933). It would appear, therefore, that Janssens' chiasmatype theory is essentially valid.

Belling (1928), Sax (1932) and Darlington (1935) later extended and specified the chiasmatype theory, which in its present version goes by the name 'partial chiasmatype theory'. The extension specifies the time of strand breakage and the manner of reunion. Breakage is thought to occur during pachytene of meiosis I after synapsis has been completed, followed by non-sister chromatid reunion.

Intergenic and Intragenic Crossing-over

The development of selective methods by which rare mutational and recombinational events could be studied led to the discovery of intragenic recombination in the 1950's. Since intergenic and intragenic recombination each have unique features, it may be worthwhile to summarize briefly the pertinent basic facts about intergenic and intragenic recombination.

Intergenic crossing-over is predominantly reciprocal; that is, there

is a 1:1 relationship between the two complementary crossover chromatids and the two parental, or non-crossover, chromatids. In addition, intergenic crossover is characterized by what Muller (1916) termed 'interference' in order to account for his observation that the occurrence of one crossover in *Drosophila* interferes with the coincident occurrence of another in the same pair of chromosomes. The phenomenon has subsequently been termed 'positive interference' in order to distinguish it from 'negative interference' which signifies the tendency of crossovers to occur in clusters.

Although the chiasmatype theory can explain the symmetrical nature of intergenic crossing-over, no satisfactory explanation for the occurrence of positive interference between crossovers and how and what causes apparent breaks in intact structures, has been provided. Darlington's (1935, 1937) 'torsional hypothesis' attempts to provide some answer to the latter question. It implies a coincidence in time of breakage and chromosome division which is thought to result in a repulsion of the 'relationally coiled' homologues, thus generating a torsion and consequent breakage. But subsequent demonstrations that chromosome division (i.e., the production of a pair of nucleoprotein fibrils from a single one that gives rise to chromatids) as seen at the earliest in pachytene closely follows chromosome replication in the interphase (Taylor, 1965) would suggest that chromosome division and breakage would be unlikely to be coincident events. Belling's (1931, 1933) replication hypothesis, in contrast, states that strand exchange could occur without strand breakage. The hypothesis, however, also requires that chromosome replication and crossing-over must be simultaneous events; furthermore, it predicts a conservative mode of chromosome replication during pachytene. Therefore, from what is known at the present time about the nature and time of chromosome replication in higher organisms,

the Belling hypothesis is no longer considered valid.

Attempts have been made to relate positive interference to mechanical phenomena but these appear far from satisfactory and, likewise, the accompanying explanations for chromosome breakage are far from clear. In recent years, suggestions have been made correlating chromosome breakage with DNA breakage. In view of the fact that enzymes are known that could cause breaks in the DNA molecule and that DNA appears to be the material responsible for the integrity of the chromosome (Callan and Macgregor, 1958), such suggestions would appear reasonable. However, the precise manner by which such enzymes might be involved in the process of recombination is not understood at the present time.

Intragenic crossing-over, in contrast with intergenic crossing-over, is characterized by negative interference, non-reciprocal crossing-over and polarization. Using selective methods and a chromosome region marked by outside markers it was discovered that intragenic recombination is often accompanied by additional exchange in close proximity, resulting in unexpected end-marker combinations. Pritchard (1955) first observed the phenomenon within the *ad-8* locus of *Aspergillus nidulans* in a study of mitotic recombination, and soon it was demonstrated to be a general feature of meiotic intragenic recombination in *Neurospora crassa*, *Aspergillus nidulans* and *Saccharomyces*.

Contrary to the regular 1:1 segregation of allelic pairs during meiosis, in intragenic recombination it was found that a non-reciprocal type of recombination involving aberrant segregation also occurs (Mitchell, 1955a, b). In the following years, this non-reciprocity was demonstrated in several species of fungi, and the term 'conversion' was generally applied to this to distinguish it from reciprocal recombination.

Some further interesting discoveries concerning intragenic recombination were made by Lissouba and Rizet (1960) using *Ascobolus immersus*. They made use of spore color mutants which, by appropriate crosses, they could subdivide into several 'series'. When different markers of the same series were crossed and the aberrant tetrads analyzed, it was found that the same one of the two markers was almost always the site of non-reciprocal recombination while the other segregated normally. Furthermore, it was noted that the marker giving non-reciprocal recombination lay either always to the left or always to the right of the normally segregating locus. They concluded that the frequency of conversion shows a continuous increase from one end of the gene to the other. They proposed the term 'polaron' to denote the genetic region with such directed conversion. Polarity in intragenic crossing-over has also been demonstrated in *Neurospora* (Murray, 1963) and in *Aspergillus* (Siddiqi and Putrament, 1963). Some of the features of intragenic recombination have also been demonstrated in *Drosophila* (Hexter, 1963; Chovnick *et al.*, 1970) and maize (Nelson, 1962).

Hypotheses to Explain Recombination

Efforts to understand intragenic crossing-over in relation to classical notions about the recombination of linked genes have led to a number of models. To account for the high negative interference, Pritchard (1955) proposed his hypothesis of effective pairing, seen as a pre-pachytene association which is intimate and localized. This event does not occur in all cells at a given location, but rather in only a fraction of them. This intense physical association between segments is thought to cause high negative interference. According to the hypothesis, positive interference

would result from spacing of the effective pairing regions, which would be variable in position.

The different recombination hypotheses are generally based on two different premises concerning the recombination mechanism: that of breakage-and-reunion and that of copy-choice, respectively. Recombination models based on copy-choice do not appear to be valid, particularly in view of the findings concerning chromosome replication, which is semi-conservative and occurs during the interphase when the chromosomes are not in homologous association.

Whitehouse (1963) proposed a model based on breakage-and-reunion of DNA molecules (chromatids) followed by the formation of hybrid DNA in the region of the breaks. Events postulated in his polaron hybrid DNA model are as follows: A single strand breaks in one of the two DNA double helices of each member of a homologous pair. The two broken strands then separate from their complementary strands on one side of each break. On the other, DNA replication proceeds on the unbroken strands to fill the gap resulting from the separation of the strands. These newly-synthesized strands then separate from their templates. The four resultant single strands then reassociate by complementary base pairing in a crosswise configuration reminiscent of a chiasma. Finally, the filling of gaps in the duplex crossover molecules by complementary nucleotides and the breakdown of unpaired non-crossover strands is postulated in order to give the completed crossover.

The Whitehouse model seems to explain satisfactorily the bulk of the data on intragenic recombination. It can neatly explain the origin of aberrant tetrads since the hypothesis postulates that the site of a mutational difference will sometimes happen to lie within the hybrid region,

where the hybrid DNA will be heterozygous and depending on the correction of heterozygosity or lack of it, different tetrad segregations may result. Negative interference would, likewise, result from correction of mispairing in the DNA at a mutant site because such correction would cause recombination between the mutant and the neighboring markers on both sides, thus giving rise to double-crossovers which are 'essentially single events in origin'. In other words, recombination by correction of heterozygosity at one point along a chromosome would favor recombination in its immediate vicinity. Polarity in intragenic recombination is thought to be the consequence of the occurrence of primary breakages in DNA at specific sites, the fixed opening points, which define the ends of a polaron (Hastings and Whitehouse, 1964). And if there is variability in the length of the hybrid DNA segment from the origin then mutant sites near the origin will occur in the hybrid DNA more often than those further away, resulting in a gradient of conversion frequency with distance from the origin.

Although the Whitehouse model would appear to be compatible with the features of recombination, there still remain some questions to be resolved. For example, it is not certain what features of the DNA determine the site for primary breakage; that is, whether or not there is a specific sequence in the DNA that may play a role in the process. Again, the precise mechanism of the repair of mispaired bases in the hybrid DNA is no known, although the situation may apparently look analogous to the excision of thymine dimers from DNA of UV irradiated cells (Boyce and Howard-Flanders, 1964). Finally, although breakage in the DNA molecule and subsequent generation of DNA single strands have been conceived as being enzyme-regulated, other possibilities such as DNA denaturation and delayed replication of a chromosome region would also appear to play a role

in generating DNA single strands.

Recently, Alberts and Frey (1970) have made some interesting observations on the gene 32 product of T_4 phage which may have some bearing on hybrid DNA models of genetic recombination. Both Whitehouse's (1963) and Holliday's (1964) models implicate DNA denaturation and renaturation in the region of hybrid DNA formation. The 32-protein was found to be one of the principal DNA-binding proteins. It caused denaturation of poly dAT at 25°C, and evidence was presented which indicates that by virtue of a strong selective affinity for single-stranded DNA, it accelerates renaturation under physiological conditions by forcing the single strands into an unfolded conformation which leaves their bases available for pairing during chance collisions between complementary strands. It is thus possible that the 32-protein may be involved both in generating single-stranded regions in the DNA molecule and in facilitating the mating of two DNA single strands to generate hybrid DNA regions.

The implication of DNA in models of crossing-over places some significance on recent findings concerning DNA synthesis during meiotic prophase. Hotta *et al.* (1966) demonstrated a small amount of DNA synthesis which occurs during the zygotene and pachytene stages of meiosis in cultured microsporocytes of *Lilium*. In a later study, Hotta and Stern (1971) found evidence that the zygotene DNA replicates semiconservatively, and using DNA-DNA hybridization techniques they also noted that the zygotene DNA synthesis represents a delayed replication. Furthermore, they have observed that the pachytene DNA synthesis does not have the characteristics of a semiconservative replication and suggested that it may represent repair replication. Although the precise functions of the zygotene and pachytene DNA synthesis are not known, the authors favor the idea that in

some unknown way the zygotene DNA may be related to the process of chromosome pairing and the pachytene DNA to the formation of chiasmata. Recently S.M. Chiu (personal communication) using phenethyl alcohol, an inhibitor of the initiation of DNA synthesis, found evidence in *Chlamydomonas reinhardi* that regions of delayed replication probably consist of whole replicons.

The Synaptinemal Complex

Ever since the discovery of the synaptinemal complex (Moses, 1958) associated with the chromatin during synapsis, and its location within the paired homologous chromosomes, it has been speculated that the structure may be involved in homologous pairing and genetic exchange. The possibility has been confirmed by Meyer (1961, 1964) who demonstrated that where genetic exchange does not take place no synaptinemal complex occurs (in normal *Drosophila melanogaster* males and in *Drosophila melanogaster* $C_{(3)}G$ homozygous females with suppressed crossing-over).

The synaptinemal complex is thought to be laid down in the stem of the 'Y' where the early zygotene homologues are joined in a bivalent. The structure consists of two 'lateral elements' each ranging from 300 to 1000 Å in thickness and each presumed to represent the 'axial core' of the single meiotic prophase chromosome. Lying between the lateral elements, a 'central element' occurs which is generally less dense than the lateral elements and appears to be of the order of 200 Å in width. Fine filaments appear to traverse the central region in which the central element lies. In *Lilium* pollen mother cells the central element is occasionally seen as two parallel strands, each about 25 Å thick and each of which is sometimes seen as a pair of fine strands of 10 to 15 Å each (Moens, 1968).

The chemical composition of the synaptinemal complex is quite uncertain at the present time. Attempts made to localize DNA in it have shown

that DNA is scarce or absent in the central region or central element but present together with protein in lateral elements (see review by Moses, 1968). Regarding the presence and localization of RNA in the synaptinemal complex there seems to be no unequivocal evidence. Moses (1968) evaluating reports on the protein composition of the synaptinemal complex tends to favor the contention that the lateral elements contain proteins, some of them basic and histone-like in character.

Many workers have observed the termination of the axial core against the nuclear membrane. The classical concept of synapsis is that it occurs in a zipper-like process which may start at the ends or the centromeres of the chromosomes. The significance of the termination of the synaptinemal complex against the nuclear membrane is not clearly understood but such association may, nevertheless, imply that in some organisms at least, synapsis initiates at the ends of the chromosome.

Although the functions of the synaptinemal complex are not well understood at present, some generalization can, nevertheless, be made. The statements provided by Moses (1968) beautifully summarize the possible conclusions that can be drawn regarding its relationships with meiotic events.

"(a) Synaptinemal complex is neither responsible for nor a consequence of pairing itself (viz. absence in somatic pairing, including the close pairing in pachytene chromosomes, and in some achiasmate pairing).

"(b) Synaptinemal complex is neither responsible for nor a consequence of chromosome reduction, which occurs in achiasmate forms lacking synaptinemal complex.

"(c) Synaptinemal complex is not essential to recombination itself (viz. not present in prokaryotes, somatic crossing over) but is required for the more regular and extensive exchange at meiosis.

"(d) Synaptinemal complex is dependent on the production of a precursor substance (protein), other than or in excess of the usual chromosomal constituents, occurring characteristically in meiosis, the formation of which is under genetic control.

"(e) The following requirements for crossing over may be served by synaptinemal complex: to provide highly specific and close pairing (effective synapsis), to hold pairing fixed for an appreciable time, to provide a limited structural framework within which recombination at the molecular level may occur, and to separate the DNA concerned with recombination from the bulk of the DNA."

The Effects of Various Treatments and Conditions on Crossing-over

A number of kinds of treatments and conditions have been found to influence the apparent rate of crossing-over in organisms with nucleoprotein chromosomes. The frequency of recombination remains constant only in experiments which are carried out under the same conditions. Following is a brief summary of the factors that have been found to affect crossing-over.

(a) *Genetic factors*. In many organisms different wild type stocks have shown different recombination frequencies under constant environmental conditions (*Saccharomyces cerevisiae*, Desborough and Lindegren, 1959; *Schizophyllum commune*, Raper *et al.*, 1958; *Coprinus lagopus*, Day, 1958).

Considerable heterogeneity in recombination frequencies also exists in crosses of *Neurospora crassa* when different strains are used (see Frost, 1961).

(b) *Temperature.* Meiotic processes are known to be influenced by temperature. Esser and Kuenen (1967) have summarized some of the innumerable results obtained with fungi. The post-reduction (indicating crossing-over between a gene and the centromere) frequency of many genes changes with a change in temperature and the change in post-reduction frequency is not uniform for the different markers studied; in some cases it increases, in others it decreases both as the temperature is raised or as it is lowered. The dependence of post-reduction frequency upon temperature is generally non-linear and a clearly defined maximum or minimum is rare. Esser and Kuenen (1967) also compiled the data on different fungi and presented them graphically to show that genetic markers according to their position on the chromosome, show a change in post-reduction frequency which is characteristic for all organisms when the temperature is lowered from the optimal (25—28°C) to 15°C.

Increased temperatures have been found to have an enhancing effect on intergenic crossing-over in *Drosophila* (Plough, 1921), *Ustilago* (Huttig, 1931), *Ascobolus* (Lissouba *et al.*, 1962). In some cases temperature sensitivity seems to be particularly strong in the vicinity of the centromere (*Neurospora crassa*, Rifaat, 1959; Towe and Stadler, 1964; *Drosophila*, Plough, 1921). In *Ascobolus*, Lissouba *et al.* (1962) found that both intergenic and intragenic crossing-over are differentially affected by temperature.

(c) *Chromosomal anomalies.* Chromosomal aberrations have a marked effect on recombination. In *Drosophila*, for example, it has been found that in inversion heterozygotes the rate of intergenic crossing-over is

higher in chromosomes not involved in the structural anomaly. Suzuki (1962, 1963) studied the effects of various X-chromosomal anomalies on chromosome III of *Drosophila* and found that these anomalies were associated with increased recombination in chromosome III. Several hypotheses have been proposed to explain these interchromosomal effects (Mather, 1936; Schultz and Redfield, 1951; Oksaka, 1958; Suzuki, 1962); but none seem to be satisfactory.

(d) *Chemical treatments.* Many ions (including Ca^{++}, Mg^{++}, Mn^{++}, K^{+}, phosphate) and the chelating agent EDTA, have been used in the study of recombination and chiasma frequency. The approach seems to have been initiated by the hypothetical chromosome structure proposed by Mazia (1954) who viewed the chromosome as composed of macromolecular complexes linked together by divalent ions. Eversole and Tatum (1956) found that EDTA and $MnCl_2$ increased recombination in *Chlamydomonas reinhardi*. The effect of manganese may be by the replacement of magnesium by manganese. Levine (1955) observed reduced recombination in *Drosophila melanogaster* raised in medium with high calcium while Paliwal and Hyde (1957) observed that a deficiency of calcium and magnesium increased chiasma frequency. Law (1963) studied the effect of potassium on recombination in the X-chromosome of *Drosophila melanogaster*. He found that potassium produced dwarf flies in which recombination was significantly higher than in the controls. The potassium-effect could not be detected in treated but normal flies suggesting that the process controlling dwarfing also controlled crossing-over. It would thus seem that potassium acts on recombination by production of dwarf flies and subsequent increase in recombination. Bennett and Rees (1970) found phosphate to cause an increase in chiasma frequency in rye.

Huttig (1933) carried out an extensive study of the effect of chemicals on intergenic crossing-over in *Ustilago*. He noted that ammonium nitrate, thiocyanate and sulfate show the same optimal concentration for increase in crossing-over. He also found that the potassium and sodium cations enhanced crossing-over. Similarly, urethane derivatives markedly increased crossing-over.

(e) *Irradiation*. There are many instances which indicate that the recombination process is sensitive to radiation. However, observations on the radiation effects on crossing-over do not offer any clear explanation of the mechanism. Stadler (1928) found that X-rays decrease crossing-over in maize. In *Drosophila*, however, many experimenters report that radiation has an enhancing effect on crossing-over (see, for example, Whittinghill and Davis, 1961, for both ionizing and non-ionizing radiation). This is explained by Westergaard (1964) as resulting from 'somatic, gonial, pre-meiotic rather than meiotic crossing-over'. Esser and Kuenen (1967) compiled a list of fungi relating the alterations of meiotic and mitotic recombination frequencies caused by UV, X and gamma radiation. It is seen that while all these radiation treatments alter mitotic recombination frequencies, the meiotic recombination frequency remains unaltered. Thus, although it is fairly well established that radiation affects mitotic recombination (the underlying mechanism involved is not understood), the question of radiation effects on meiotic recombination is yet to be resolved.

Studies on the effects of chemicals and treatments on recombination have mostly been carried out using non-synchronized systems. The use of synchronized systems in such studies, however, is expected to provide a better understanding of these effects. Such studies with *Chlamydomonas*

reinhardi have been reported by Lawrence (1965), Lawrence and Davies (1967) and Davies and Lawrence (1967).

Genetic Control of Recombination

Evidence in the higher plants that genetic control may be implicated in the mechanism of crossing-over comes from the occurrence of meiosis-specific genes. These genes are generally of two types, asynaptic and desynaptic. Beadle (1930) reported an asynaptic gene in maize on chromosome 1 causing a drop of chiasma frequency per bivalent from 1.7 to 0.5, and Prakken (1943) described an asynaptic factor in rye which appeared to be controlled by a single recessive gene. In addition, several desynaptic genes have been reported (for example, in *Hordeum vulgare, Oryza sativa* and *Triticum aestivum;* see Riley and Law, 1965). Recently evidence has come to light which suggests genetic control at later stages of meiosis. Rhoades and Dempsey (1966) reported a factor in maize which they designated as *elongate (el)* because the factor appeared to despiralize the chromosome at both meiotic anaphases. The gene is believed to suppress the first division, allowing the second to proceed normally with the resultant production of half tetrads.

Gene mutations that alter recombination are also known in *Drosophila.* The gene $C_{(3)}G$ (Gowen and Gowen, 1922) at 57.4 on the right arm of chromosome 3 reduces recombination, when homozygous, to less than one percent of the normal. Recently Sandler *et al.* (1968) have reported several other genes in *Drosophila melanogaster* that affect one or more of the meiotic processes.

Recent studies in fungi have provided some interesting cases implicating gene involvement in the control of recombination. In 1965, Jessop and Catcheside reported the occurrence of a gene in *Neurospora crassa* which

enhanced recombination between alleles of the *his-1* locus by a factor of ten. That this effect was controlled by a single locus was demonstrated in the clear segregation of *rec*$^+$ and *rec*$^-$ and by the existence of classical dominance-recessive relationship between the two alleles, *rec*$^+$ being dominant. Smith (1966) described a second recombination gene in *N. crassa* that affected recombination between two non-allelic markers, *pyr-3* and *leu-2*, which flank the *his-5* locus. In the presence of this gene, recombination between the two non-allelic markers is enhanced from 9.9 percent to 21.5 percent. As in the previous case, simple segregation patterns point to a single gene governing this behavior. Although such evidence implies that gene products may be involved in the control of crossing-over, it is not known whether these are RNA, protein or, in fact, substrate metabolites.

MATERIALS AND METHODS

Two aberration-free high viability stocks were used in the study. One stock, obtained from the Department of Genetics, University of Wisconsin, had the markers *y cv v f*; the other was a wild type Oregon-R stock from the Department of Biology, Yale University. Crossing-over was measured in the X-chromosome using the markers: *y* = yellow body, 0.0; *cv* = crossveinless, 13.7; *v* = vermilion eye, 30.0 and *f* = forked bristles, 56.7 (see Bridges and Brehme, 1944). The region covered included most of the euchromatin but did not extend into the heterochromatin. The three segments *y—cv*, *cv—v* and *v—f* were designated 1, 2 and 3, respectively (see Ting and Walker, 1969; Walker and Bradley, 1969).

A semidefinitive culture medium (Nash and Bell, 1968) was used in the experiments. This consisted of 10 gm yeast, 10 gm sucrose, 1 gm agar, 1 ml propionic acid and 10 mg chloramphenicol in 100 ml distilled water. The stock cultures and crosses were incubated in 1/4-pint bottles containing about 25 ml culture medium, at a constant temperature of 25 ± 1°C.

A standard testcrossing procedure was carried out as follows: In each bottle four virgin *y cv v f / y cv v f* females were mated with four Ore-R *+ + + +* / Y males for four days ± 1 hour, to obtain tetrahybrid flies. In order to achieve an optimum moisture level inside the bottle, a perforated piece of "Parafilm" was wrapped over the plug and sealed along the rim of the bottle. When tetrahybrid flies emerged, four virgin females were isolated from each treatment bottle and mated in a bottle with four tester males in the manner outlined above.

When a substantial number of testcross flies had emerged in any bottle (usually by the 14th - 15th day after initiation of the cross) they

were collected and promptly classified (this is in contrast with the procedure of accumulating the flies in a freezer, as followed by Walker and Bradley [1969] for comparable experiments). This offered considerably greater ease in handling and classifying the flies, with the operation spreading over a period of 2—4 days at the end of which emergence was complete.

Flies were classified and scored for genotype and sex. Data were then tabulated with respect to exchange chromosomes according to the notations: SE_1, SE_2, SE_3 = single-exchange in segments 1, 2, and 3, respectively; $DE_{1,2}$, $DE_{1,3}$, $DE_{2,3}$ = double-exchanges involving segments 1 and 2, 1 and 3, and 2 and 3, respectively; TE = triple-exchange; OE = no exchange; SE_T = single-exchange total and DE_T = double-exchange total.

Tetrahybrid flies were raised either in semidefinitive medium (control) or in semidefinitive medium plus urethane, selenocystine or sodium monohydrogen arsenate (referred to subsequently as arsenate) obtained from Koch-light, Calbiochem and Fisher Scientific Company, respectively.

Three experiments were performed, designated A, B, and C, each of them replicated as two separate treatment series designated I and II. Experiment A was performed using urethane alone in the concentrations: 0 mM, 0.5 mM, 2 mM, 3 mM, 4 mM, 10 mM and 25 mM. In experiment B, urethane and selenocystine were used in 16 different treatment combinations and in experiment C urethane and arsenate were used in 20 different treatment combinations (the term 'treatment combination' refers to a combination of specific concentrations of the two treatment chemicals in one treatment). The treatments are listed below (where U, Se and As symbolize urethane, selenocystine, and arsenate, respectively; the numerals indicate concentra-

tions in mM for urethane and in μM for selenocystine and arsenate).

Experiment A

0 U, 0.5 U, 2 U, 3 U, 4 U, 10 U and 25 U

Experiment B

* (0 U + 0 Se) (0 U + 2 Se) (0 U + 10 Se) (0 U + 50 Se)
* (1 U + 0 Se) (1 U + 2 Se) (1 U + 10 Se) (1 U + 50 Se)
* (5 U + 0 Se) (5 U + 2 Se) (5 U + 10 Se) (5 U + 50 Se)
*(10 U + 0 Se) (10 U + 2 Se) (10 U + 10 Se) (10 U + 50 Se)

Experiment C

* (0 U + 0 As) (0 U + 2 As) (0 U + 10 As) (0 U + 50 As) (0 U + 100 As)
* (1 U + 0 As) (1 U + 2 As) (1 U + 10 As) (1 U + 50 As) (1 U + 100 As)
* (5 U + 0 As) (5 U + 2 As) (5 U + 10 As) (5 U + 50 As) (5 U + 100 As)
*(10 U + 0 As) (10 U + 2 As) (10 U + 10 As) (10 U + 50 As) (10 U + 100 As)

In order to assess the effects of the chemicals on exchange frequencies, analyses of variance were carried out on the percentages of each of the scoring classes. To illustrate, the percentages of exchange for segment 1 (SE_1) for the seven concentrations of treatment chemical in experiment A were arranged as shown below and the analyses were carried out in the usual manner.

Exchange class	Series	Concentration of urethane, mM							Total
		0	.5	2	3	4	10	25	
SE_1	I	4.90	5.08	5.98	5.77	5.78	5.80	4.6	37.91
	II	3.94	5.01	5.48	5.92	5.39	5.77	5.12	36.63
Total		8.84	10.09	11.46	11.69	11.17	11.57	9.72	74.54

---

*A single set of treatments was used to provide data for both experiments.

Similar analyses were performed to assess the effects of urethane and selenocystine in experiment B and arsenate in experiment C. The treatment combinations from experiment B and C isolated for these analyses are as follows:

Urethane (experiment B): 0 U + 0 Se, 1 U + 0 Se, 5 U + 0 Se,
10 U + 0 Se.

Selenocystine (experiment B): 0 U+ 0 Se, 0 U + 2 Se, - U + 10 Se,
0 U + 50 Se.

Arsenate (experiment C): 0 U + 0 As, 0 U + 2 As, 0 U + 10 As,
0 U + 50 As, 0 U + 100 As.

In addition, analyses of variance were performed to test for interactions between urethane and selenocystine in experiment B and between urethane and arsenate in experiment C. These analyses were done, as in the previous cases, for each exchange class on the entire range of treatment combinations within the experiment. The general procedure followed in arranging the data for analysis is shown below, using as an example the exchange class SE_1 in experiment B.

Series	Se µM / U mM	0	2	10	50	Total
I	0	4.79	6.27	4.64	6.22	21.92
II		4.84	5.88	4.56	6.25	21.53
	Sum	9.63	12.15	9.20	12.47	43.45
I	1	5.54	5.38	5.32	4.14	20.38
II		3.89	5.20	5.41	4.01	18.51
	Sum	9.43	10.58	10.73	8.15	38.89
I	5	5.94	5.85	4.56	5.78	22.13
II		6.68	7.10	4.43	5.37	23.58
	Sum	12.62	12.95	8.99	11.15	45.71
I	10	3.96	5.50	3.10	5.47	18.03
II		5.15	5.74	3.81	4.62	19.32
	Sum	9.11	11.24	6.81	10.09	37.35
Total		40.79	46.92	35.83	41.86	165.40

Analysis of variance has also been performed after converting the percentages into angles (angle = arc sin $\sqrt{\text{percentage}}$). See footnotes on pages 37, 38 and 42.

Graphical Presentation of the Data

For the graphical presentation of the data, paired replicates were pooled and the frequency of each exchange class so derived was plotted against treatment-chemical concentration. This pooling appears to be justified by the existence of an acceptable degree of homogeneity in the replicates (see Figs. 1, 2, 3).

Treatment frequencies for each exchange class were transformed before plotting to the form:

$$\frac{\text{Estimated frequency for a specific treatment}}{\text{Corresponding estimate from total data}}$$

These transformations permit a clearer comparison of effects on different curves than do the simple treatment frequencies, since all treatment points are expressed as comparisons with a relatively stable 'total set'. The following worked example (using values marked '*' in Table I) will illustrate the general procedure for deriving graph points from individual-treatment data.

$$SE_1 \text{ (for 0 mM U; Table 1)} = \frac{54 \quad + \quad 52}{1102 + 1317} \Big/ \frac{975}{17{,}975} = 0.86$$

OBSERVATIONS AND RESULTS

Physiological Effects of Treatment

Observations and assessments for possible deleterious physiological effects of treatment on the tetrahybrids were made on each bottle for:

(1) length of time between initiation of the culture and the emergence of the first fly,

(2) larval and pupal mortality,

(3) yield, both on the total and on male and female emergence, and

(4) adult abnormalities.

In experiment A, for concentrations up to 10 mM urethane, no major differences from controls were detected except for total yield at 10 mM urethane, which was 90% of the control. At 25 mM urethane a marked delay in male first emergence of 5 days beyond first-fly emergence (12th day) was observed. Examinations on day 20 and during incubation of the bottles for an additional period of two weeks revealed that there was also extensive male pupal lethality. Of a total of 473 adult flies only 23 were male and of 438 non-eclosed pupae all were male. It is therefore evident that while female yield is not affected by this concentration of urethane, the treatment causes a 95% male pupal mortality. The female flies appeared quite normal; the males looked slightly smaller than normal, probably due to retarded growth.

In experiment B, no deleterious effects of any treatment combination were observed. In experiment C, 100 μM arsenate caused a 16% drop in yield as compared with the control. Apart from this no other physiological effects of high arsenate treatment were detected.

Scoring Regularity in Test Cross Progeny

A close parity was found between sexes in the total data for all exchange classes in all experiments except OE (OE males were less frequent than OE females in all experiments). Similarly, an examination of total data with respect to pairs of reciprocal crossover subclasses revealed a close approximation to a 1:1 ratio for all exchange (crossover) classes in all experiments.

The scoring data of experiments A, B and C with sexes and reciprocal crossover subclasses combined are presented in Tables I, II and III, respectively, where number of flies in different exchange classes are shown against different treatments. Since TE flies are very small in number and since the analysis concerns itself with chromosome exchange events rather than recombination values this exchange class was not used in further analysis.

Breakdowns of the data in Tables I, II and III with respect to the two bottle-series are shown in Figs. 1, 2 and 3, respectively. Here the correlations between the two series with respect to the adjusted number of flies in the single-exchange, double-exchange and no-exchange groups are presented as scatter diagrams. Each exchange-class value for a series has been adjusted for a total fly population equal to the average of the two series. Paired values represent the point co-ordinates and the limit lines are the loci for $\chi^2 = 3.8$. (Note that where two or more identical value pairs occurred, these were represented by a single point, so that in some instances the number of graph points is somewhat lower than the expected number.)

Clustering within the significance boundaries is consistent with

Table 1

Scoring data from experiment A; sexes and reciprocal crossover subclasses combined.

Urethane Concentration mM	Series	Scoring classes								Total
		SE_1	SE_2	SE_3	$DE_{1,2}$	$DE_{1,3}$	$DE_{2,3}$	TE	OE	
0	I	54*	169	206	1	12	16	0	644	1102*
	II	52*	155	227	3	13	20	0	847	1317*
5	I	55	165	187	6	10	29	1	628	1081
	II	56	185	209	4	18	25	0	620	1117
2	I	67	172	206	4	16	12	2	641	1120
	II	58	150	196	3	14	12	1	624	1058
3	I	81	258	297	3	22	33	0	709	1403
	II	96	305	321	8	23	44	0	823	1620
4	I	109	307	369	2	33	47	2	1014	1883
	II	95	303	347	6	21	31	1	958	1762
10	I	79	222	263	4	12	37	2	743	1362
	II	80	226	290	4	15	44	2	725	1386
25	I	39	88	170	1	2	7	1	539	847
	II	47	105	174	1	4	16	0	570	917
All treatments		975*	2820	3472	50	215	373	12	10078	17975*

*Values used in the worked example of materials and methods demonstrating the procedure followed to calculate relative frequencies.

Table II

Scoring data from experiment B; sexes and reciprocal crossover subclasses combined.

Treatment		Series	Scoring classes								Total
U mM	Se µM		SE_1	SE_2	SE_3	$DE_{1,2}$	$DE_{1,3}$	$DE_{2,3}$	TE	OE	
0	0	I	64	177	234	4	15	28	1	806	1334
		II	71	232	263	3	12	36	0	848	1465
1	0	I	71	218	230	2	10	20	0	730	1281
		II	62	232	332	0	14	42	0	910	1592
5	0	I	98	272	338	7	30	41	1	861	1648
		II	108	287	313	7	25	47	0	829	1616
10	0	I	67	294	341	2	20	38	0	928	1690
		II	69	209	287	3	17	35	0	719	1339
0	2	I	83	200	286	5	20	31	0	697	1322
		II	84	230	308	0	19	27	0	759	1427
1	2	I	57	181	212	1	13	19	0	575	1058
		II	51	172	194	2	10	23	0	527	979
5	2	I	73	194	255	3	13	34	0	680	1247
		II	88	216	246	3	11	26	0	648	1238
10	2	I	80	244	318	0	15	38	0	759	1454
		II	83	257	310	0	24	38	0	733	1445
0	10	I	73	233	294	1	15	35	1	920	1572
		II	61	219	258	1	11	31	0	756	1337

(Cont'd)

Table II (Cont'd)

Treatment		Series	Scoring classes								Total
U mM	Se µM		SE_1	SE_2	SE_3	$DE_{1,2}$	$DE_{1,3}$	$DE_{2,3}$	TE	OE	
1	10	I	82	245	299	2	29	46	0	837	1540
		II	72	228	270	2	19	35	0	704	1330
5	10	I	73	242	303	2	11	24	1	942	1598
		II	69	258	300	2	30	29	0	869	1557
10	10	I	49	243	304	0	21	29	0	931	1577
		II	56	238	286	1	19	29	0	837	1466
0	50	I	92	251	290	0	22	33	0	789	1477
		II	88	228	270	1	21	25	0	775	1408
1	50	I	69	249	323	1	20	35	0	967	1664
		II	57	225	313	4	21	26	0	772	1418
5	50	I	70	201	215	0	8	28	1	687	1210
		II	85	291	297	2	23	35	0	849	1582
10	50	I	90	287	336	5	23	41	0	862	1644
		II	70	259	297	1	18	39	1	830	1515
All Combinations			2365	7512	9127	67	579	1043	6	25367	46030

TABLE III

Scoring data from experiment C; sexes and reciprocal crossover subclasses combined.

Treatment		Series	Scoring classes								Total
U mM	As µM		SE_1	SE_2	SE_3	$DE_{1,2}$	$DE_{1,3}$	$DE_{2,3}$	TE	OE	
0	0	I	64	177	234	4	15	28	1	801	1334
		II	71	232	263	3	12	36	0	848	1465
1	0	I	71	218	230	2	10	20	0	730	1281
		II	62	232	332	0	14	42	0	910	1592
5	0	I	98	272	338	7	30	41	1	861	1648
		II	108	287	313	7	25	47	0	829	1616
10	0	I	67	294	341	2	20	38	0	928	1690
		II	69	209	287	3	17	35	0	719	1339
0	2	I	77	212	310	3	18	28	1	835	1448
		II	95	200	264	4	17	27	0	726	1333
1	2	I	55	167	229	1	10	32	0	684	1178
		II	62	169	268	1	15	21	0	643	1179
5	2	I	86	264	288	2	17	42	0	901	1600
		II	73	246	323	3	15	31	0	854	1545

(Cont'd)

Table III (Cont'd)

Treatment		Series	Scoring classes								Total
U mM	As µM		SE_1	SE_2	SE_3	$DE_{1,2}$	$DE_{1,3}$	$DE_{2,3}$	TE	OE	
10	2	I	71	230	293	2	24	32	1	876	1529
		II	57	240	311	2	21	32	0	909	1572
0	10	I	110	261	301	5	21	27	0	901	1626
		II	87	228	274	2	15	36	0	760	1402
1	10	I	81	211	273	1	14	32	0	821	1433
		II	77	216	283	2	11	28	1	807	1425
5	10	I	65	259	294	3	12	26	0	888	1547
		II	89	283	282	3	21	35	0	797	1510
10	10	I	54	218	286	1	9	25	0	777	1370
		II	56	224	288	4	14	24	0	792	1402
0	50	I	80	242	264	5	12	28	0	736	1367
		II	96	236	260	6	25	35	0	746	1404
1	50	I	66	217	238	3	20	38	1	704	1287
		II	68	188	257	3	17	36	0	647	1216

(Cont'd)

Table III (Cont'd)

Treatment		Series	Scoring classes								Total
U mM	As µM		SE_1	SE_2	SE_3	$DE_{1,2}$	$DE_{1,3}$	$DE_{2,3}$	TE	OE	
5	50	I	79	260	327	5	22	29	1	1007	1730
		II	80	259	327	5	24	30	0	922	1647
10	50	I	72	237	280	0	16	31	1	889	1526
		II	75	253	287	3	19	33	0	851	1521
0	100	I	91	245	247	8	11	34	0	783	1419
		II	75	217	248	5	19	33	2	745	1344
1	100	I	89	217	245	5	30	50	1	634	1271
		II	80	189	247	4	19	34	0	691	1264
5	100	I	71	214	272	4	17	28	0	806	1412
		II	69	221	262	2	19	27	0	783	1383
10	100	I	91	264	300	2	24	49	0	834	1564
		II	86	230	258	3	17	38	0	764	1396
All Combinations			3073	9228	11229	130	708	1318	11	32139	57851

Figure 1. Scatter diagram showing the correlation between bottle series I and II in scoring values. The three single-exchange (SE_1, SE_2, SE_3), three double-exchange ($DE_{1,2}$, $DE_{1,3}$, $DE_{2,3}$) and the non-exchange scores for each treatment are included.

*Adjusted number of flies.

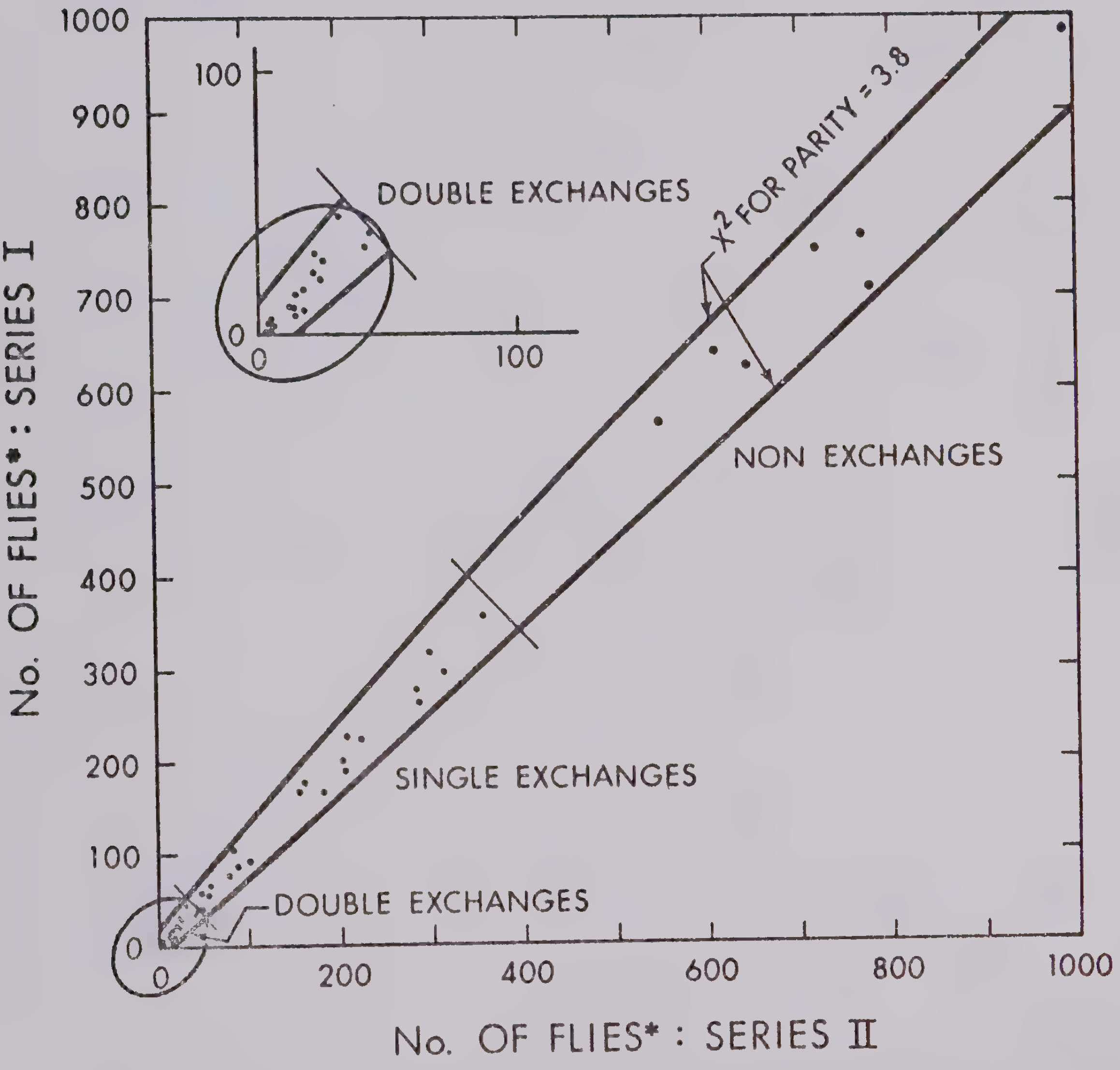

Fig. 1. Scatter diagram for experiment A.

Figure 2. Scatter diagram showing the correlation between bottle series I and II in scoring values. The three single-exchange (SE_1, SE_2, SE_3), three double-exchange ($DE_{1,2}$, $DE_{1,3}$, $DE_{2,3}$) and the non-exchange scores for each treatment are included. Arrows against dots indicate values lying outside the confidence limit.

*Adjusted number of flies.

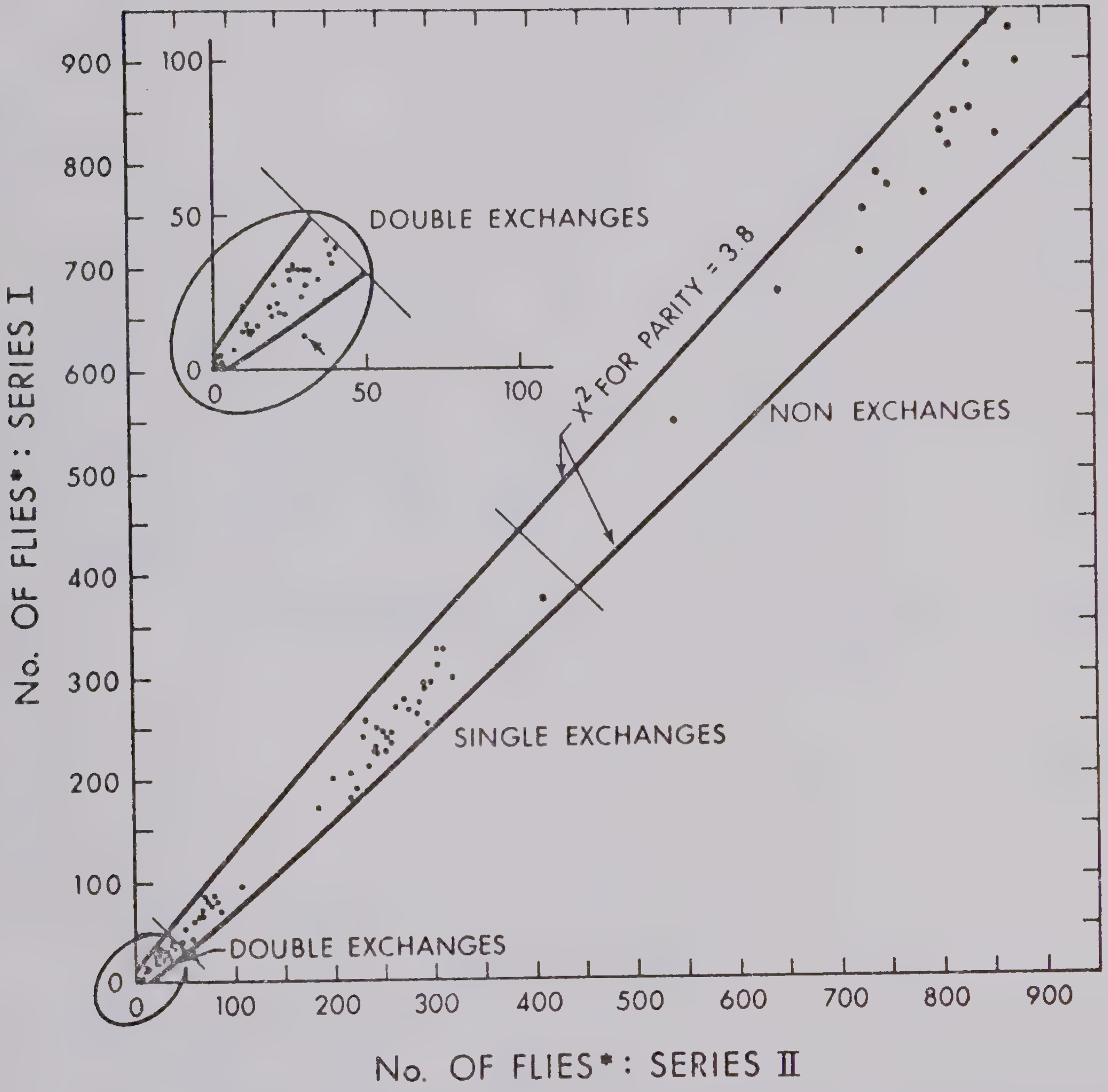

Fig. 2. Scatter diagram for experiment B.

Figure 3. Scatter diagram showing the correlation between bottle series I and II in scoring values. The three single-exchange (SE_1, SE_2, SE_3), three double-exchange ($DE_{1,2}$, $DE_{1,3}$, $DE_{2,3}$) and the non-exchange scores for each treatment are included. Arrows against dots indicate values lying outside the confidence limit.

*Adjusted number of flies.

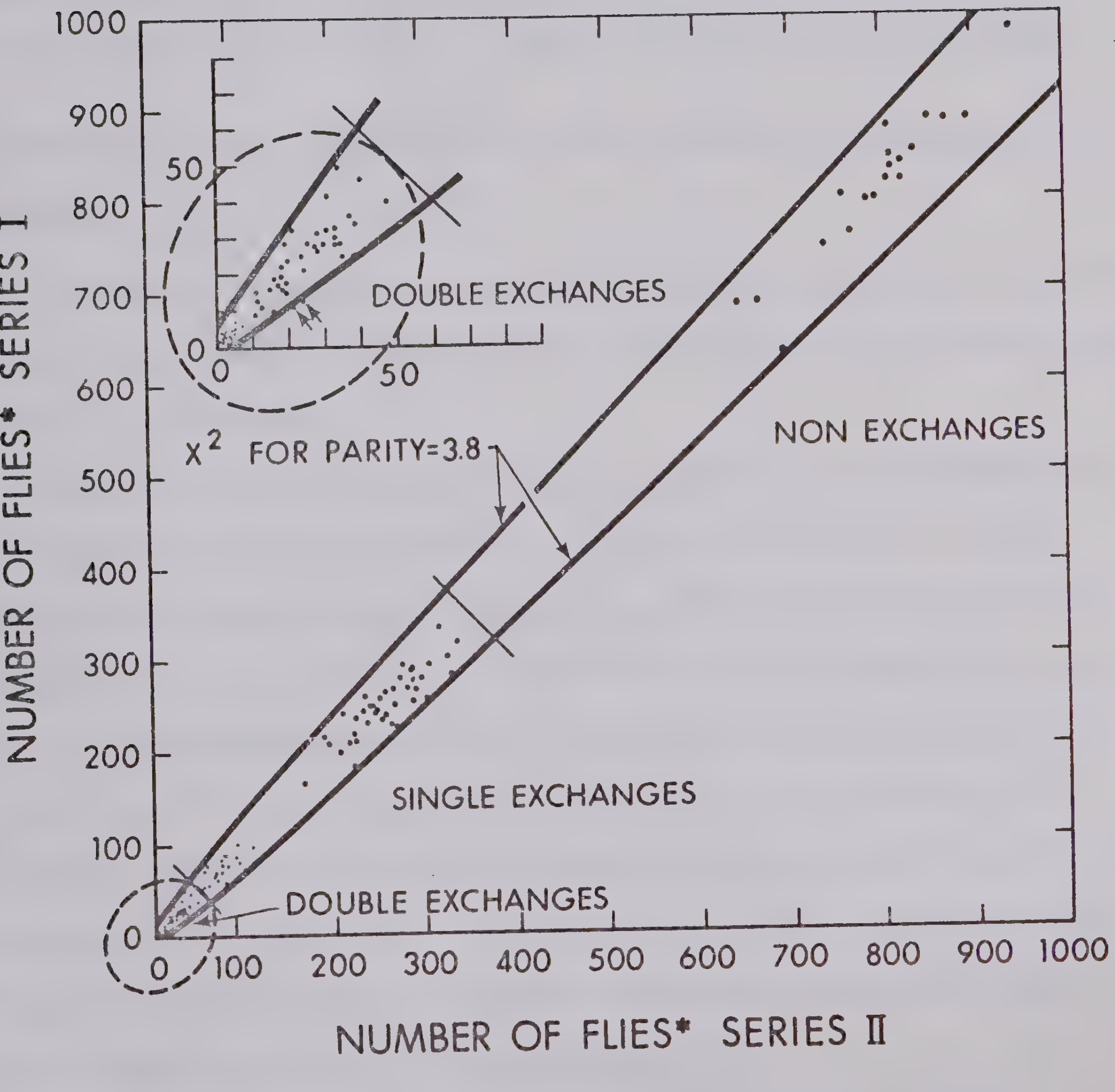

Figure 3. Scatter diagram for experiment C.

the expectation for random variation. The only value-pairs that differ significantly are: experiment B - $DE_{1,3}$ for 5 mM U 10 μM Se; experiment C - $DE_{1,3}$ for 5 mM U 10 μM As and $DE_{1,3}$ for 0 mM U 50 μM As. These values (indicated in the figures by arrows) are infrequent enough (3 out of a total of 301 such values) and close enough to the limits to be considered random variants. Thus it may be inferred that the study is relatively free of non-random distortions, not caused by the treatments themselves.

The Effects of Urethane, Selenocystine and Arsenate on the Exchange-class Frequencies

Analyses of variance were performed in order to assess the independent effects of each chemical on crossing-over according to the procedure outlined in Materials and Methods.

Results of the analyses of variance of the two sets of urethane data are presented in Tables IV-A and IV-B. It is seen in Table IV-A that except for the exchange classes SE_3 and $DE_{1,2}$ all exchange classes reveal significant treatment effects. In Table IV-B, however, significant treatment effects are found only for $DE_{1,3}$, SE and OE. With the exception of $DE_{2,3}$ in Table IV-B where replication MS appears to be rather high, the disagreements between the two sets of data do not seem to have resulted from differences in the replicates. However, the significant treatment effects on SE_T and OE found in both sets of data would suggest that the chemical has real effects, at least on single exchanges.

Analyses of variance of the selenocystine data are presented in Table V and those of the arsenate data in Table VI. The selenocystine analyses indicate high significance for single exchanges in segment 1 and segment 3 and for double exchange involving these two segments. All other

Table IV-A

Analyses of variance in percentage-frequency per scoring class with differing concentrations of urethane in experiment A. Degrees of freedom: Urethane 6, Replication 1, Error 6.

Exchange Class	MS			F (Urethane)
	Urethane	Replication	Error	
SE_1	·6091	·117	·1157	5·26*
SE_2	12·3081	·0772	1·4836	8·29*
SE_3	2·1001	·0292	·8144	2·57
$DE_{1,2}$	·028	·0092	·0149	1·87
$DE_{1,3}$	·3299	·0023	·0756	4·36*
$DE_{2,3}$	·9944	·0321	·1567	6·34*
OE	38·6398	·312	4·2735	9·04**
SE_T	24·5079	·6301	3·9544	6·19*
DE_T	2·0187	·1063	·2357	8·56*

*Significant at 5% level
**Significant at 1% level

Table IV-B

Analyses of variance in percentage-frequency per scoring class with differing concentrations of urethane in experiment B. Degrees of freedom: Urethane 3, Replication 1, Error 3.

Exchange Class	MS			F (Urethane)
	Urethane	Replication	Error	
SE_1	1·327	·0136	·7769	1·70
SE_2	2·4475	·021	2·8805	·84
SE_3	3·3073	1·4706	1·4207	2·32
$DE_{1,2}$	·0386	·0004	·0046	8·39
$DE_{1,3}$	·2824	·0221	·0241	11·71*
$DE_{2,3}$	·1273	·616	·0593	2·14
OE	20·7477	2·5538	·6194	33·49**
SE_T	14·6362	1·4028	1·4916	9·81*†
DE_T	1·1237	·3828	·1248	9·00

*Significant at 5% level
**Significant at 1% level
†This was found nonsignificant in the analysis after angular transformation.

Table V

Analyses of variance in percentage-frequency per scoring class with differing concentrations of selenocystine in experiment B. Degrees of freedom: Selenocystine 3, Replication 1, Error 3.

Exchange Class	MS			F (Selenocystine)
	Selenocystine	Replication	Error	
SE_1	1.4207	.019	.0206	68.96**
SE_2	1.395	2.322	1.06	1.31
SE_3	5.1732	.03	.1117	46.31**
$DE_{1,2}$	.0196	.0181	.0189	1.03
$DE_{1,3}$	.189	.0465	.0087	21.72*
$DE_{2,3}$	.0338	.0265	.0864	.39
OE	16.4529	.7321	1.9542	8.41
SE_T	16.0297	2.42	1.7425	9.16†
DE_T	.0844	.2701	.1057	.79

*Significant at 5% level

**Significant at 1% level

†This was found significant in the analysis after angular transformation.

Table VI

Analyses of variance in percentage-frequency per scoring class with differing concentrations of arsenate in experiment C. Degrees of freedom: Arsenate 4, Replication 1, Error 4.

Exchange Class	MS			F (Arsenate)
	Arsenate	Replication	Error	
SE_1	.8815	.2497	.6537	1.34
SE_2	2.9711	.219	1.0992	2.70
SE_3	2.1561	.0372	.5079	4.24
$DE_{1,2}$	.0231	.0079	.0084	2.75
$DE_{1,3}$	.0383	.1145	.1454	.26
$DE_{2,3}$	.064	.3649	.0544	1.17
OE	8.909	3.588	.5626	15.83*
SE_T	8.6122	1.3542	1.3414	6.42*
DE_T	.1521	.7344	.144	1.05

*Significant at 5% level

exchange classes are non-significant and, moreover, the consistently low error against which they are assessed supports the view that effects, if they are present, are greatly reduced. Thus, it appears highly probable that selenocystine has differential effects, with high intensity restricted to segments 1 and 3.

The effects of arsenate on individual single-exchange and double-exchange classes does not seem to be strong, and significant F values are obtained only for OE and SE_T. This would tend to indicate that arsenate exerts a low but real cumulative effect on crossing-over along the *y—f* region.

The Interaction of Urethane with Selenocystine and Arsenate on Exchange-classes Frequencies

Tables VII and VIII show the results of analyses of variance performed to test whether urethane-selenocystine and urethane-arsenate interactive effects on specific exchange-class frequencies are present. F values for interactions were obtained by dividing the interaction MS by the error MS. (The additional set of F values for urethane, selenocystine and arsenate included in these tables for reference, were derived by dividing the treatment MS by the interaction MS.)

Urethane and selenocystine show significant interaction in their effects on the frequencies of exchange classes SE_1, SE_3, $DE_{2,3}$, OE, SE_T and DE_T. Exchange classes showing significant urethane-arsenate interactions are SE_1, $DE_{1,2}$, OE, SE_T and DE_T.

Effect of the Chemicals on Exchange Pattern

Figures 4a and 4b present the urethane curves with no selenocystine

Table VII

Summary of analyses of variance performed on the percentage-frequencies of exchange classes using data in experiment B to assess the interaction of urethane and selenocystine. Degrees of freedom: Urethane 3, Selenocystine 3, Interaction 9, Error 16.

Exchange Class	MS				F
	U	Se	U x Se	Error	U x Se
SE_1	1.8946	2.5862	.9558	.248	3.85**
SE_2	2.3389	1.3802	1.1628	1.0784	1.07
SE_3	2.1205	3.8227	1.7969	.6967	2.57*
$DE_{1,2}$	.0192	.0334	.0187	.0096	1.94
$DE_{1,3}$	.0139	.027	.2056	.0999	2.05
$DE_{2,3}$	.0579	.0754	.2749	.0955	2.87*
OE	4.5796	19.0678	9.8761	2.0968	4.71**
SE_T	3.5577	19.5085	6.027	1.7912	3.36*
DE_T	.1287	.0832	.7542	.2599	2.90*

*Significant at 5% level
**Significant at 1% level

Table VIII

Summary of analyses of variance performed on the percentage-frequencies of exchange classes using data in experiment C to assess the interaction of urethane and arsenate. Degrees of freedom: Urethane 3, Arsenate 4, Interaction 12, Error 20.

Exchange Class	MS				F
	U	As	U x As	Error	U x As
SE_1	2·7486	·9277	1·0475	·4021	2·60*
SE_2	1·6549	1·9975	1·6852	·8384	2·01
SE_3	2·1327	1·7554	1·2977	1·1196	1·15
$DE_{1,2}$	·059	·0284	·0157	·0057	2·75*
$DE_{1,3}$	·0278	·2162	·1716	·0809	2·12†
$DE_{2,3}$	·3097	·5011	·2811	·1532	1·83
OE	1·5071	2·7288	10·3087	1·7132	6·01**
SE_T	·4829	·7544	6·0929	1·6206	3·75†
DE_T	·2211	1·6468	·8811	·3101	2·84*

*Significant at 5% level
**Significant at 1% level
†These values were found nonsignificant in the analysis after angular transformation

Figures 4a and 4b. Effects of urethane concentration on the relative frequencies of the exchange classes. Log urethane concentration occupies the abscissa; relative frequencies are given to the left of the graph. The region on the left of the curves is presented by a dotted line since real urethane concentration for '0 mM urethane' treatment is not known. Asterisks indicate a statistically significant treatment effect at 5% (*) and 1% (**) levels.

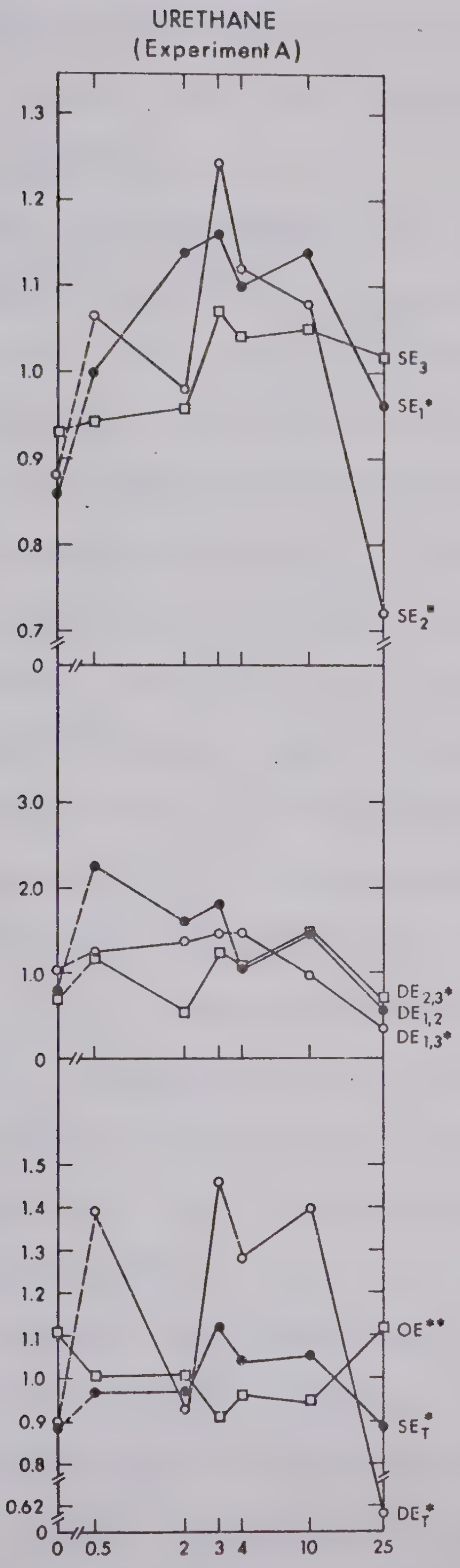
URETHANE
(Experiment A)
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0
SE3
SE1*
SE2*
3.0
2.0
1.0
0
DE2,3*
DE1,2
DE1,3*
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.62
0
OE**
SET*
DET*
0
0.5
2
3
4
10
25
LOG CONCENTRATION OF URETHANE IN mM

Fig. 4a

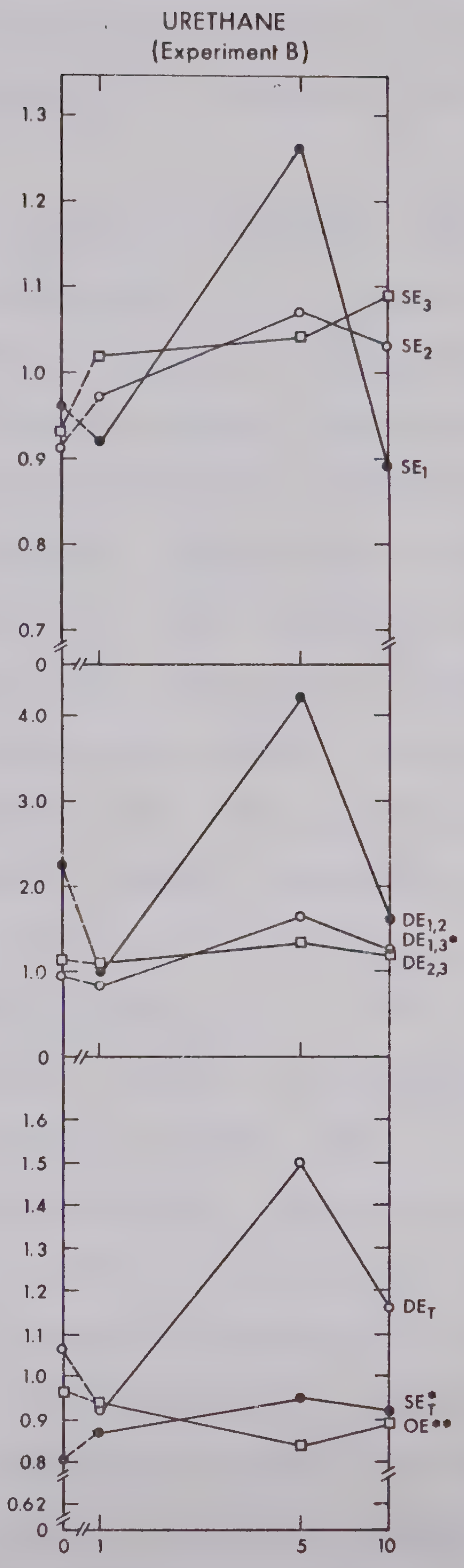
URETHANE
(Experiment B)
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0
SE3
SE2
SE1
4.0
3.0
2.0
1.0
0
DE1,2
DE1,3*
DE2,3
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.62
0
DET
SET*
OE**
0
1
5
10

Fig. 4b

or arsenate, obtained from experiments A and B, respectively. Figures 5 and 6 show the selenocystine and arsenate curves, respectively, with no urethane. Figure 5 was derived from the data of experiment B, and 6 from those of experiment C.

The curves were examined with respect to their general shape and for inflectional agreements existing between them. When a part of a curve deviated to the left of the extrapolated line from the preceding part of the curve it was said to be positively deflected, and when towards the right, to be negatively deflected. When two curves were both positively or both negatively deflected at a given point, (deflectional) concordance was said to exist between them at that point. Curves deflected to the right and left, respectively, were said to be (deflectionally) discordant.

When the urethane curves in Figure 4a are examined in this manner it is found that the three single-exchange curves show concordance at all points except one (SE_1 at 2 mM urethane). Similarly, concordance is seen in the three double-exchange curves, except for the point for $DE_{1,3}$ at 4 mM urethane. The SE_T and DE_T curves show concordance at all points. Such strong point concordances as these strongly reinforce the validities of concordant sections of the several individual treatment curves.

Curves for those exchange classes that showed statistically significant treatment-effects are peaked in overall contour, and show considerable evidence of a superimposed central peak at 3 mM urethane and two other peaks at 0.5 and 10 mM, respectively. The optimum urethane concentration range for the overall peaking effect appears to be between 3 and 10 mM urethane. Segment 3, as revealed by the SE_3 curve, while concordant with other single exchange curves, is much less responsive to urethane treatment.

Before making any comparisons with Fig. 4b, it must be pointed out

Figure 5. Effects of selenocystine concentration on the relative frequencies of exchange classes. Log selenocystine concentration occupies the abscissa; relative frequencies are given to the left of the graph. The region on the left of the curves is represented by a dotted line since real selenocystine concentration for '0 μM Se' treatment is not known. Asterisks indicate a statistically significant treatment effect at 5% (*) and 1% (**) levels.

Figure 6. Effects of arsenate concentration on relative frequencies of exchange classes. Log arsenate concentration occupies the abscissa; relative frequencies are given to the left of the graph. The region on the left of the curve is represented by a dotted line since real arsenate concentration for ' 0 μM As' treatment is not known. Asterisks indicate a statistically significant treatment effect at 5% (*) and 1% (**) levels. The arrow in the $DE_{1,3}$ curve indicates the point which lies outside the confidence limit in the scatter diagram (Fig. 3).

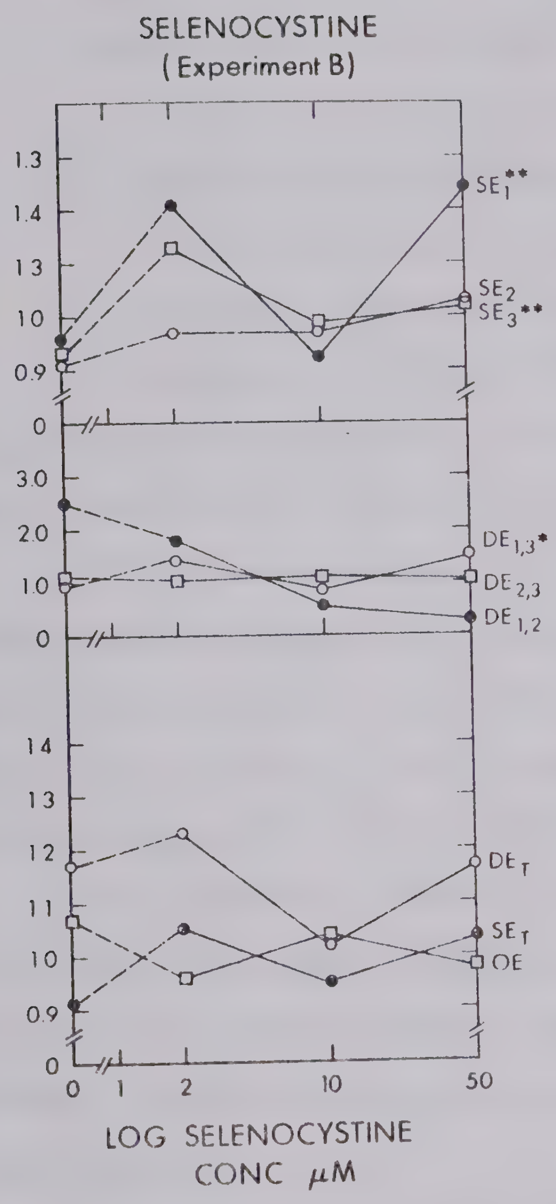
SELENOCYSTINE
(Experiment B)
1.3
1.4
1.3
1.0
0.9
0
3.0
2.0
1.0
0
1.4
1.3
1.2
1.1
1.0
0.9
0
SE_1^{**}
SE_2
SE_3^{**}
$DE_{1,3}^*$
$DE_{2,3}$
$DE_{1,2}$
DE_T
SE_T
OE
0
1
2
10
50
LOG SELENOCYSTINE CONC μM

Fig. 5

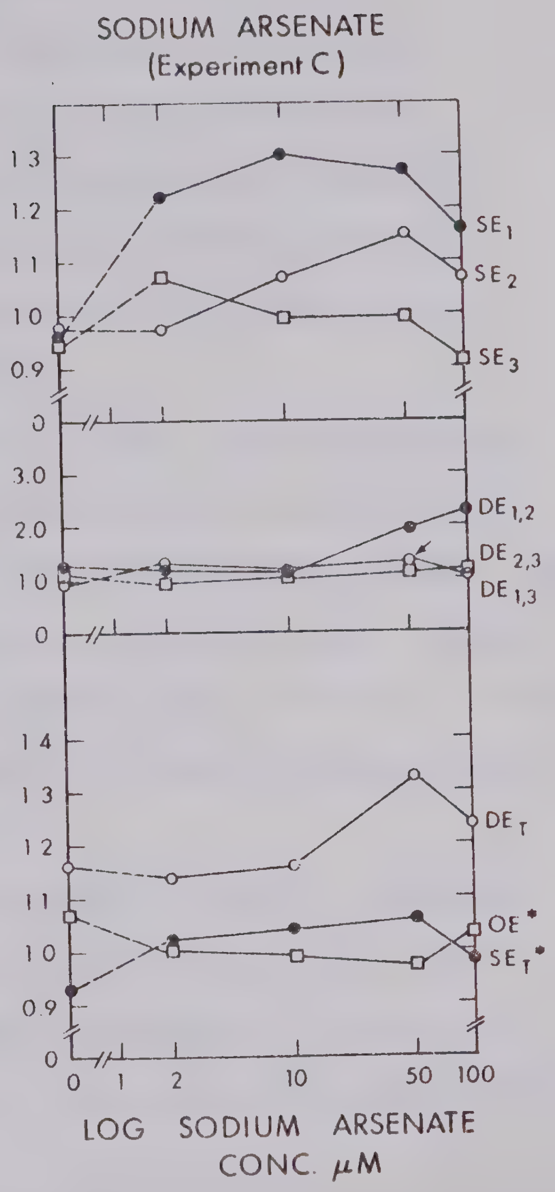
SODIUM ARSENATE
(Experiment C)
1.3
1.2
1.1
1.0
0.9
0
3.0
2.0
1.0
0
1.4
1.3
1.2
1.1
1.0
0.9
0
SE_1
SE_2
SE_3
$DE_{1,2}$
$DE_{2,3}$
$DE_{1,3}$
DE_T
OE^*
SE_T^*
0
1
2
10
50
100
LOG SODIUM ARSENATE CONC. μM

Fig. 6

that the denominator of the transformation values for the common treatment points 0 mM and 10 mM urethane in the two sets of data can differ according to:

(1) differences in the dried yeast batch used,

(2) differences in the experimental design (thus the total frequency per exchange class will, if urethane has an appreciable effect, differ in the two sets of data because in experiment A there are seven concentrations while in experiment B there are only four, of which only two are common to the two sets of data).

Thus, the urethane curves of Fig. 4b do not reveal the overall peak and trough characteristics of the Fig. 4a curves as might be expected since the intermediate concentrations, 2, 3, and 4 mM are not represented. Instead, a peak appears here in all curves except SE_3 at 5 mM urethane. The high degree of concordance in these curves indicates that it represents a real effect and, in addition, it falls within the optimal concentration range for the overall peak in Fig. 4a curves.

It is concluded, therefore, that urethane treatment induces an overall peak in exchange frequency at an optimum concentration close to 3 mM. The response is greatly reduced in segment 3 compared to the other two segments. In addition, it is noted that there are secondary peaks in the Fig. 4a curves.

In the selenocystine curves of Fig. 5 it is seen that those for SE_1, SE_3, SE_T, DE_T, and $DE_{1,3}$ are each doubly inflected with a 'peak' at 2 μM Se and a 'trough' at 10 μM Se. These curves are concordant at all treatment points. In addition, the SE_2 and $DE_{1,2}$ curves, although not showing a pronounced peak and trough, nevertheless show complete concordance

with those having these features. The single exception to high concordance (other than OE) is $D_{2,3}$ which is discordant with most of the other curves at all points.

It would thus appear that the peak and trough features of the selenocystine curves are real. Highly significant treatment effects revealed by the analysis of variance on curves where these features are most pronounced (SE_1, SE_3, and $DE_{1,3}$) contribute further to the validity of these features.

The two selenocystine concentration optima appear to lie at 2 and 50 μM Se. At 10 μM Se the level of response to the treatment appears to be the same as that of control for SE_2, $DE_{1,3}$, and SE_T and slightly lower than the control for SE_1, DE_T, and $DE_{1,2}$.

In the arsenate curves, the most marked responses are found in SE_1 and $DE_{1,2}$, which may be seen to influence the SE_T and DE_T curves that follow very similar contours and are highly concordant with SE_1 and $DE_{1,2}$, respectively. SE_2 and SE_3 show some point discordance with SE_1, but it can be seen that the curve for their average $(\frac{SE_1 + SE_2}{2})$ would show reasonably high concordance. On the other hand, $DE_{1,3}$ and $DE_{2,3}$ are relatively inert to arsenate, and show little evidence of concordance with $DE_{1,2}$.

The effect seen is an overall 'peaked' response for single-exchange and a 'trough-and-peak' response for double-exchange, superimposed on a general increase in both groups over the treatment range. Both the 'peaked' and the 'trough-and-peak' responses appear to result entirely from treatment effects on segment 1 and, to a less marked extent, segment 2.

Figures 7 and 8 present the selenocystine and arsenate families of curves for specific urethane concentrations. The selenocystine and the arsenate curve with 0 mM urethane may be considered as the respective basic

Figure 7. Effects of selenocystine and urethane concentration on the relative frequencies of exchange classes. Log selenocystine concentration occupies the abscissa; relative frequencies are given to the left of the graphs. The region on the left of the curves is represented by a dotted line since the real (low trace) selenocystine concentration for '0 µM Se' treatment is not known. The arrow in one of the $DE_{1,3}$ curves indicates the point which lies outside the confidence limit in the scatter diagram (Fig. 2). Statistically significant interaction at 5% level has been denoted by an asterisk (*) and that at 1% level by two asterisks (**).

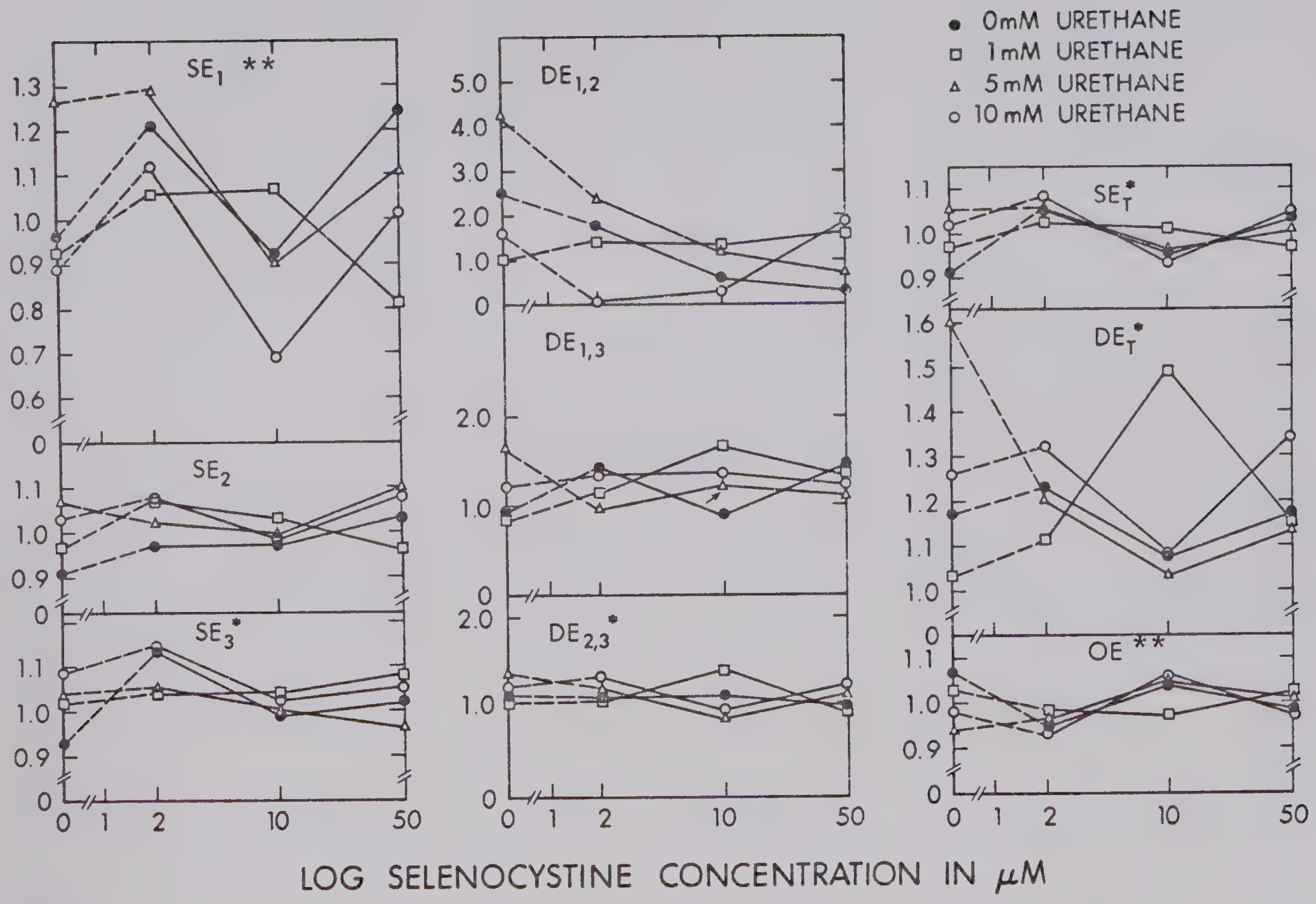
● 0mM URETHANE
□ 1mM URETHANE
▵ 5mM URETHANE
○ 10mM URETHANE
SE1 **
SE2
SE3*
DE1,2
DE1,3
DE2,3*
SET*
DET*
OE **
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0
5.0
4.0
3.0
2.0
1.5
1.4
1.6
0 1 2 10 50
LOG SELENOCYSTINE CONCENTRATION IN μM

Fig. 7

Figure 8. Effects of arsenate and urethane concentration on the relative frequencies of exchange classes. Log arsenate concentration occupies the abscissa; relative frequencies are given to the left of the graphs. The region on the left of the curves is represented by a dotted line since real (low trace) arsenate concentration for '0 µM As' treatment is not known. The arrows in two of the $DE_{1,3}$ curves indicate points which lie outside the confidence limit in the scatter diagram (Fig. 3). Statistically significant interaction at 5% and 1% levels has been denoted by asterisks (*) and (**), respectively.

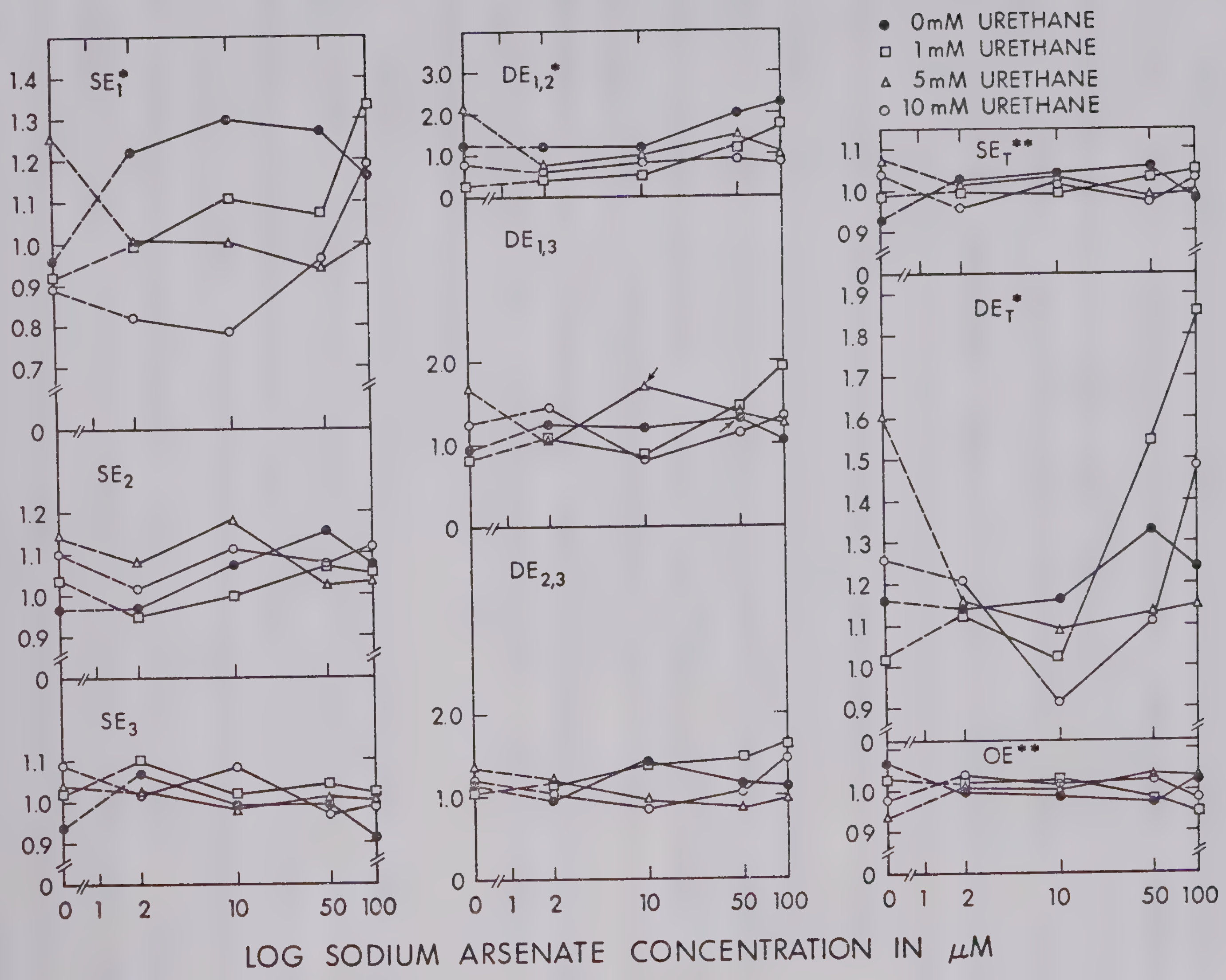
● 0mM URETHANE
□ 1mM URETHANE
△ 5mM URETHANE
○ 10 mM URETHANE
SE_1^*
SE_2
SE_3
$DE_{1,2}^*$
$DE_{1,3}$
$DE_{2,3}$
SE_T^{**}
DE_T^*
OE^{**}
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0
3.0
2.0
1.9
1.8
1.7
1.6
1.5
0 1 2 10 50 100
LOG SODIUM ARSENATE CONCENTRATION IN μM

Fig. 8

response curves for these chemicals. The corresponding curves for different urethane concentrations may then be compared with these in order to assess the nature of urethane-selenocystine and urethane-arsenate interactions.

Several manifestations of interaction are conceivable in curves of this nature. First, curves occurring in parallel arrays would indicate an absence of interaction, since they would signify that the basic pattern of response to the first chemical had not been affected by urethane. Second, an accentuation of inflectional changes in the basic response curve caused by urethane would indicate synergism in the sense that urethane magnifies the basic response. Third, if in contrast, these changes are decimated by urethane, the interaction would be suppressive. Fourth, where the inflectional changes in the basic response curve are reversed, the interaction would be antagonistic. Finally, if the inflectional change in the basic response curve is different in different parts of the curve, this would indicate a form of interaction suggesting that concentration effects of either or both chemicals are themselves complex. This situation may be classed as complex interaction.

Examining those sets of curves in Fig. 7 which showed statistical significance for interaction, it may be seen that in SE_1, SE_T, OE, and DE_T (except for a possible anomaly at 0 µM Se 5 mM U) the selenocystine curves for urethane concentrations 5 and 10 mM show complete concordance with the basic response curve for all points. In SE_3 a similar tendency is present but partly obscured by possibly anomalous values. It is not clear, however, whether the effects of 5 and 10 mM urethane are synergistic, neutral, or suppressive. In contrast, the $DE_{2,3}$ curves for these urethane concentrations are predominantly discordant, suggesting antagonism (this is also true of $DE_{1,2}$ and $DE_{1,3}$, which, however, did not show significance).

A very marked and consistent urethane effect is, however, seen at 1 mM urethane. The effect, clearly noticeable in all the curves which showed statistically significant interaction, consists of a very marked inflectional reversal of the basic response curves at 10 μM Se and, in addition, a suppression of the deflectional change in the basic response curve at 2 μM Se. Thus the interacting effect of 1 mM urethane may be said to be either complex (suppressive-antagonistic) or predominantly antagonistic. In this respect, the possibility may be pointed out that the difference between apparent suppression and antagonism may be only of degree of effect.

The arsenate curves in Figure 8 which, according to significance estimates, would be expected to show indifferent effects, nevertheless show some unexpected but clear interactive features. In the SE_1 set of curves, those for 1 and 5 mM urethane follow, with some exceptions, the basic response curve for arsenate up to 50 μM arsenate, after which both the curves provide evidence of strong antagonistic interaction. The curve for 10 mM urethane represents a complete reversal of the basic arsenate response curve which indicates an overall antagonistic interaction. In $DE_{1,2}$, however, the curve for 1 mM urethane closely follows the basic response curve, while those for 5 and 10 mM urethane seem to reverse the inflections in the basic response curve in two of the three points where such inflectional changes can be examined. In SE_T the curve for 1 mM urethane closely follows the basic response curve except for the reversal at 50 μM arsenate, while those for 5 and 10 mM urethane appear to show reversal of the basic response curve at all points. In DE_T the 1 and 10 mM urethane curves are each antagonistic at two points and synergistic at one point, while that for 5 mM urethane does not provide much evidence of

antagonism. In the OE family of curves, all three curves (for 1, 5, and 10 mM urethane) appear to indicate antagonistic interaction.

In general, therefore, it would seem that the interaction between urethane and arsenate is predominantly antagonistic and is strongest at 10 mM urethane for all levels of arsenate. These basic antagonistic effects furthermore generally agree in that the antagonism is progressively greater with increasing urethane concentration.

DISCUSSION

The results of these experiments indicate that urethane has a marked effect on crossing-over in the X-chromosome of *Drosophila melanogaster*. Significant data from experiment A reveal that urethane varied exchange frequencies for all classes except SE_3 and $DE_{1,2}$ and those from experiment B indicate an effect on the exchange classes SE_T, DE_T, and OE. Despite some disagreement in the two sets of urethane data there is, nevertheless, considerable agreement in the nature of the two sets of curves. Therefore, although the features in the curves of experiment A are much less evident in those of experiment B, the disagreements are not expected to affect the conclusions reached on the features of the urethane curves, namely, the presence of a concentration optimum for urethane characterized by increased crossing-over. In general, the urethane curves show this concentration optimum to range from 3 to 10 mM.

This confirms reports by Huttig (1933) working with *Ustilago* species that this chemical enhances crossing-over. In addition the urethane curves are of the same general shape as those found by Huttig (for recombination in the region between the mating type locus and the centromere): exchange increases to a maximum level at an optimum urethane concentration and decreases past this level, so that two opposed sub-effects appear to be operating.

It is possible that this situation may reflect what is currently known about the biochemical effects and the metabolic fate of urethane *in vivo*. McKinney (1950) noted that ethyl urethane at a concentration of 10 mM inhibited the methylation of nicotinamide and glycocyamine, and this observation led Boyland and Koller (1954) to suggest that it should also inhibit

the biosynthesis of thymine, the methyl group of which is introduced by transmethylation. If so, it is conceivable that urethane might produce a deficiency of thymidine for DNA and hence might act directly to produce DNA lesions during major synthesis or repair synthesis in the meiotic prophase. However, this possibility has not been experimentally tested.

On the other hand, Boyland and Nery (1965) have shown in several mammals that urethane is oxidized by a process of N-hydroxylation to produce N-hydroxyurethane which has been demonstrated by Freese (1967) to react readily with oxygen, producing H_2O_2 and (free) radicals. Freese (*ibid.*) also demonstrated with *Bacillus subtilis* that hydroxyurethane inactivates transforming DNA, while urethane has no such effect. In view of these observations and the reports for higher plants (Oehlkers, 1943), *Drosophila* (Vogt, 1948) and Walker rat carcinoma (Boyland and Koller, 1954) that urethane treatment induces chromosome breakage, it may be inferred that breakage via hydroxyurethane is a likely avenue for the effects of urethane treatment found in this study. Nevertheless, the failure of urethane to inactivate transforming DNA does not necessarily imply that urethane *per se* has little or no effect on meiotic chromosome integrity, since genomic DNA undergoes replication and repair whereas DNA *in situ* during inactivation does not.

Whatever the nature of urethane action may be, its effect on crossing-over is probably mediated predominantly through its involvement in the process of chromosome breakage. The reversal of the urethane curves at higher urethane concentrations might result from a combination of two things: (a) the relative concentrations of urethane vs. hydroxyurethane might be drastically reversed by rapid excretion of hydroxyurethane above a critical *in vivo* concentration; (b) urethane may be neutral in its effects or much

less active than hydroxyurethane and thus decimate breakage effects by effect-inhibition.

The selenocystine curves are doubly inflected with a 'peak' at 2 μM Se and a 'trough' at 10 μM Se. Walker and Bradley (1969) described a similar selenocystine effect on crossing-over in the X-chromosome of *Drosophila melanogaster* and the present study, therefore, confirms their observations. For the selenocystine effects, these authors offered the interpretation, based on previous reports of ready incorporation of Se in place of S into proteins (see Jaguregui-Adell, 1966, for review), that differential selenium incorporation in the chromosomal protein altered the stress characteristics of the protein on the associated DNA resulting in a differential 'propensity' for DNA breakage. It is also conceivable, however, that the effect is mediated by differentially altered activities of various enzymes that may be involved in recombination as a result of differential incorporation of selenium. An example of selenium incorporation in an enzyme resulting in an altered activity of the enzyme is provided by the work of Ahluwalia (1967) who noted a non-linear relationship between the activity of the seven-cysteine alkaline phosphatase of *E. coli* and the degree of incorporation of selenium in the enzyme. It would also appear possible that at different levels of selenocystine, effects on different components of the recombination process may influence the efficiency of recombination. If the selenocystine effect is mediated in this manner, one may not find a simple relationship between crossing-over and concentration such as the presence of one concentration optimum.

The effect of arsenate on crossing-over appears to be relatively weak; significant treatment effects were found only in the exchange classes SE_T and OE. Nevertheless, examination of the single exchange curves reveals

regulate pH and osmotic differential across the membrane and to permit the entry and exit of essential metabolites without collapse of the membrane potential. In Mitchell's (1966) hypothesis uncouplers are agents capable of degrading the structure of the membrane and eliminating the pH gradient across it. Despite the functional differences between the mitochondrial and nuclear membrane, some effect of arsenate on the latter would appear conceivable. The effects of many ions on crossing-over and chiasma frequency have been reported by many authors (see Literature Review) and the possibility that these ions may affect recombination through physiological routes has not been ruled out. In addition, the possibility that the effects reported may also have resulted from specific anionic effects (Na^+, H^+) caused by using the sodium monohydrogen salt is also not ruled out.

Rather clear indications from treatment curves for all three chemicals that the effects on segment 1 are very closely correlated with those on the double-exchange curves involving this segment are seen when concordance of the SE_1, $DE_{1,2}$, and $DE_{1,3}$ curves are compared. It has been noted that when the two sets of urethane data are combined and the resulting curves compared, the SE_1 curve shows strong inflectional agreement with those of $DE_{1,2}$ and $DE_{1,3}$. Similarly, SE_1 shows complete concordance with $DE_{1,3}$ in the selenocystine curves and fairly high concordance with $DE_{1,3}$ in the arsenate curves. Walker and Bradley (1969) found a similar inflectional agreement between the selenocystine treatment curves SE_1 and $DE_{1,3}$. The situation would tend to imply that segment 1 exerts an influence on the nature of the double-exchange curves, particularly the $DE_{1,3}$ curves.

Several manifestations of interaction between urethane and selenocystine have been noted. Of these interactions, the most conspicuous are the synergistic interaction of a high level (10 mM) of urethane with all levels of selenocystine and the antagonistic interaction of a low level (1 mM) with high levels of selenocystine (10—50 μM). The synergistic interaction would suggest that the two interacting factors act on a common mechanism of crossing-over. However, in the absence of any knowledge of the chemical interaction between urethane and selenocystine and the mode of action of the latter in the process of crossing-over, no substantial inferences can be drawn regarding the interacting effects of the chemicals on crossing-over. Thus, apart from the observation that the selenocystine curves appear to be consistent with at least a 2-factor effect and that at low urethane concentration the one factor is nullified, no chemical explanation for this phenomenon has been found.

The interesting feature of interaction between urethane and arsenate is the reversal of the arsenate curves by 10 mM urethane, suggesting an antagonistic interaction. It is difficult to explain such antagonistic interacting effects because of lack of information on their chemical interaction. However, one way to explain the antagonistic effects is by invoking a situation of some kind of complexing between urethane and arsenate. This would explain the initial downward deflection and the following upward deflection of the arsenate curve caused by a constant amount of urethane. According to the hypothesis of complex formation, the downward deflection would then represent the complexing and the upward deflection, the residual arsenate effect.

An interesting observation in the study is the lethality of the male flies grown in medium containing 25 mM urethane. The lethal effect parallels

earlier observations by Oehlkers (1943, 1952, 1956) on three higher plants, Oehlkers and Marquardt (1950) on *Peonia*, Jensen *et al.* (1951) on *Neurospora* and Vogt (1948) on *Drosophila*. It has been noted that the development of the male flies progressed very close to the time of emergence and no evidence of significant mortality of the eggs and larvae could be detected. If the lethality were due only to extensive chromosome damage caused by urethane or hydroxyurethane, then both male and female flies should have shown the response. But since there is the sex-differential in the urethane effect, the situation may imply that the X-chromosomes are involved in the response. Thus, chromosome damage followed by its subsequent effects on the relationship between the X-chromosomes and the autosomes may result in the phenomenon. In other words, the two X-chromosomes of the female may be thought to complement each other to nullify the deleterious effects of the treatment. Another possibility, namely the physiological difference between the male and female flies producing the effect, cannot also be ruled out.

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